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Regulation of salinity tolerance in *Brassica juncea* (L.) introgression lines: Osmoprotectants, antioxidative molecules and ionic content

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Abstract

Indian mustard [*Brassica juncea* (L.) Czern & Coss] is one of the most important oilseed crops worldwide yet drought and salinity stress significantly reduced its growth and yield. The research was carried out in order to test the effect of salinity on osmoprotectants (total soluble sugars, proline), antioxidant molecules (ascorbate, α-tocopherol) and ionic content from the dry sample of the leaves in introgression lines and varieties of *Brassica juncea*. Permanent saline plots are maintained in the field of soil sciences where different doses of sodium carbonate were given to maintain the relative sodium bicarbonate (RSC) to three levels. The results revealed the significant effect of salinity on biochemical attributes as well as on ionic content. Increase in total soluble sugars, proline, ascorbate, α-tocopherol and Na^+ ion whereas rest of the ions Ca^{2+}, Mg^{2+} and K^+ decreased with increased salinity levels. Increased accumulation of Na^+ increased the Na^+/K^+ ratio and decreased the K^+/Na^+. Significant finding among the introgression lines and varieties revealed low Na^+ and high K^+ correspondingly Na^+/K^+ low and K^+/Na^+ ratio. Decreased calcium and magnesium ion resulted in decline in chlorophyll content and membrane stability under saline conditions while decreased K^+ concentration regulated the opening and closing of stomata thus hampering photosynthesis.

Keywords: *Brassica juncea*; Osmoprotectants; Antioxidative molecules; Ionic content

1. Introduction

Brassicaceae or *cruciferae* is a medium sized nearly crucial family of flowering plant. Brassicas species is the most important edible oil source in the world after soybean and palm [1].In India, it is grown in 6.3 mha with production of 7.6mt and productivity of 11.90 q/ha [2]. Among the abiotic stress factors salinity is known to decrease plant growth and productivity due to ionic and osmotic stresses. Over 830 million hectares of land in the whole earth is salt affected, either through saline water (403 million hectares) or due to sodicity (434 million hectares) [3]. Salinity exerts a cumulative impact on diverse imperative elements of plant metabolism viz. mineral ion homeostasis, osmolyte accumulation, antioxidant metabolism, nitrogen fixation and photosynthetic functionality adversely affects plant growth, improvement and productivity [4]. Stress interrupt the ordinary oxidative methods that causes cell damage [5]. Osmoprotectants or compatible solutes are small organic molecules with neutral charge and low toxicity at high concentrations that act as osmolytes and help organisms survive extreme osmotic stress [6]. Under stress conditions such as drought or high salinity plants naturally produce or take up osmoprotectants which show increased survival rates. Osmoprotectants can prevent the photo system-salt interactions, reducing ROS production so total soluble sugars and proline has a key role in osmotic regulation in plants. Osmotic adjustment in plants exposed to salinity stress depends largely on soluble sugars. The enhanced sugars in response to stress possibly acts as compatible solutes in the stabilization of biological structures and the bimolecular proline protects the enzymes, stabilize the machinery of

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protein synthesis, regulation of cytosolic acidity and also acts as an effective singlet oxygen quencher. Antioxidants are compounds that inhibit oxidation. To balance the oxidative state, plants and animals maintain complex system of overlapping antioxidants such as glutathione and enzymes, produced internally or the dietary antioxidants vitamin C and vitamin E [7]. Ascorbate and α-tocopherol acts as antioxidant molecules. Ascorbic acid serves as an important cofactor for enzymes which are involved in the synthesis of collagen, carnitin and neurotransmitters [8]. Tocopherols, as antioxidants scavenge lipid peroxy radicals responsible for propagating lipid per oxidation [9].Antioxidant activity enhanced with increased salinity levels.

Large amount of soluble salts in the soil with Ca^{2+}, Mg^{2+}, Na^{+} and $Cl^{-}$are the main ions that intercept growth of crop at three levels by: high osmotic pressure of the soil solution that restrict the availability of water, constitute an imbalance between essential nutrients and generate specific ion toxicities [10].Plants have refined complex mechanism against the salinity such as osmotic orientation that usually consummates the inorganic ions uptake as well as aggregation of compatible solutes thereby optimizing the enzymes action in plants [11]. The capacity of ion accumulation in plants is related to their tolerance to salt stress. Availability of essential nutrients in most plants was generally reduced under salinity stress [12].Na^{+} and K^{+} homeostasis are crucial for plant growth and development, favored by low cytosolicNa^{+} and high cytosolic K^{+}[13]. It has been observed that nutrient assimilation especially K^{+} and Ca^{2+} is reduced in the rooting medium under high levels of NaCl, which ultimately leads to ion imbalance of K^{+}, Ca^{2+} and Mg^{2+} as compared to Na^{+}. Based on our previous studies of introgression lines and donar wild species to salt stress elaborate studies were conducted on the selected introgression lines for osmoprotectants, antioxidant molecules and ion content associated with the better performance in the permanent saline beds maintained in the fields.

2. Material and methods

2.1. Plant material and treatments

The experiment was conducted at the field and laboratories, Department of Soil Science and Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Four introgression lines i.e. JA-106, JA-108, JT-163, JT-498 of *Brassica juncea,* released variety(PBR 357) and national saline check (CS 52) were planted for two years according to recommended package and practices. The experiments were conducted in the permanent saline plots (2.0m x 2.5m= 5.0m^2) maintained in the field of Department of Soil Science where sodium carbonate was used to maintain desirable residual sodium bicarbonate (RSC) @ 0,3,6.5 and 10 meq/L in randomized block design with two replications.

2.2. Estimation of osmoprotectants

2.2.1. Total sugars

Total soluble sugars were measured [14] in which 0.1 gm of dry leaf sample was homogenized with 10 ml of 80% ethanol. The samples were centrifuged at 1000x g for 10 minutes and 0.1 ml of supernatant were mixed with 1 ml of 5% phenol and kept for 10 minutes followed by addition of 5 ml of H_2SO_4. The samples were placed in boiling water for 15 minutes, followed by cooling on ice to stop the reaction and absorbance was measured at 490 nm against reagent blank. The concentration of total soluble sugars was calculated from glucose standard (20-60 µg) run simultaneously.

2.2.2. Proline

Proline concentration was determined [15] where 0.2 g of leaf sample was homogenized in 10 ml of 3% sulphosalicylic acid and centrifuged at 3000 rpm for 10 minutes. The supernatant 2 ml was reacted with equal volume of glacial acetic acid and ninhydrin reagent (1.25 gm of ninhydrin, 30 ml of glacial acetic acid and 20 ml of 6M orthophosphoric acid). The sample was heated at 100^0C to which 5 ml of toluene was added after cooling in ice bath and its absorbance was read at 520 nm using toluene as blank.

2.3. Estimation of antioxidant molecules

2.3.1. Ascorbate

Ascorbic acid was measured by the method [16] in which 0.2 gm of dried leaves were homogenized in 4% TCA followed by centrifugation at 2500 rpm for 15 minutes and 0.2 ml of supernatant were mixed with 0.5 ml of 2% dinitrophenyl hydrazine solution followed by addition of two drops of thiourea reagent in the test tubes. The reaction mixture was boiled in water bath for 30 minutes at 50 °C.Then added 2.5 ml of 80% H_2SO_4 to the reaction mixture. The absorbance reading was taken at 520 nm against the water blank replacing the extract.

2.3.2. α-tocopherol

α-tocopherol was determined by the method [17] in which 0.2 g of dried leaf sample was homogenized in 5 ml of ethanol and then centrifuged at 8000 rpm for 20 minutes. 2.5 ml of supernatant was taken and added 1.5 ml of xylene in the test tubes. The solution was poured in the centrifuged tubes and again centrifuged at 8000 rpm for 10 minutes. Two layers were formed. 1 ml of xylene layer was taken out with a pipette in the test tube and 1 ml of α,α'- dipyridyl reagent was added and then read at 460 nm using alcohol as a blank and at 520 nm using $FeCl_3$ reagent as blank.

2.4. Ionic estimation in plant samples

2.4.1. Digestion of plant sample

It refers to the process where organic matter is destroyed by oxidation in liquid medium. Oxidation is carried out in the presence of a di-acid mixture of HNO_3 and $HClO_4$ in the ratio of 3:1.Weighed 0.5gm of dried plant material (3^{rd} and 4^{th} leaves of the main raceme) put it in the Erlenmeyer flask of 250 ml volume then added 15-20 ml of diacid mixture and mix the content of the flask by swirling. Place a funnel at the neck of the flask and keep it overnight. Then heat the flask at low temperature on hot plate. A vigorous reaction with the evolution of brown fumes of nitrous oxide set in immediately. After HNO_3 is boiled off, white fumes of $HClO_4$ starts coming out and the reaction is over with 1-2 minutes. After this raise the temperature to the fullest (around 400˚C) so that the refluxing of H_2SO_4 takes place at the base of the neck of the flask. Continue heating at this temperature till the content of the flask give a yellowish green appearance (1-2 ml of the digest is left behind). Cool the contents and dilute with 25 ml of distilled water. Transfer the solution to a 100 ml of volumetric flask and make up volume (50ml) by giving washing to the Erlenmeyer flask. Filter it through filter paper and used the filtrate for the determination of ions.

2.4.2. Estimation of sodium and potassium

Five ml of the filtrate was taken in 50 ml volumetric flask. Made the final volume up to 25 ml with distilled water. The solution was fed to the atomizer assembly in the flame photometer, the galvanometer of which has already been adjusted with the standard Na and K solutions and note down the reading.

Calculations for sodium

Weight of the plant material taken	= 0.5 g
Volume made	= 50 ml
First dilution	= 100 times
Second dilution	= 50 times
Total dilution	= 100 x 50= 5000 times
Reading shown by flame photometer	= A
Ppm of Na against A as read from the standard curve	= Y
Ppm of Na in the plant sample	= Y x 5000= B
(%) Na in the given plant sample	= $B/10^4$

Calculations for potassium

Weight of the plant material taken	= 0.5g
Volume made	= 50 ml
First dilution	= 100 times
Second dilution	= 25 times
Total dilution	= 100 x 50= 2500 times
Reading shown by flame photometer	= A
Ppm of K against A as read from the standard curve	= Y
Ppm of K in the plant sample	= Y x 2500= B

(%) K in the given plant sample = $B/10^4$

2.4.3. Estimation of calcium

Pipette out 1 ml of filterate in a volumetric flask. Diluted with 25 ml distilled water. Added2-3 crystals of carbamate and 10 drops of 4N NaOH solution. Added approximately 50mg of purpurate indicator. Stir with the help of glass rod and titrate with 0.01N versenate solution till the color gradually changed from orange red to violet (purple). EDTA should be added drop wise.

Calculations

Volume of 0.01 N EDTA solution used = X ml

Milli equivalents (me) of Ca^{++}/ litre= X x 0.01x1000/ml of extract taken

2.4.4. Estimation of magnesium

Pipette out 1 ml of filtrate in a flask. Diluted with 25 ml distilled water. Add 2-3 carbamate crystals and 2 drops of Erichrome black T indicator. Now add 10 drops of NH_4Cl-NH_4OH buffer and titrated against 0.01N EDTA solution. The end point will be change of color from wine red to blue or green.

Calculations

Volume of 0.01 N EDTA solution used = Y ml

Milli equivalents (me) of Ca^{++} + Mg^{++} per litre= Yx0.01x1000/ml of extract taken

Milli-equivalents of Mg^{++} per litre = (Ca^{++} + Mg^{++}) – Ca^{++}, both in me/L

2.4.5. Estimation of chlorides

Took 1 ml of filtrate in a clean conical flask. Add 2 drops of 1% phenolphthalein indicator. Appearance of pink color will show the presence of carbonates. Titrate it against N/50 H_2SO_4, till the solution becomes colourless. To this added 2-3 drops of methyl red indicator. Titrated it with N/50 H_2SO_4, till the colour changed from yellow to pink. Added few mg of $CaCO_3$ and few drops of the potassium dichromate indicator and titrated against standard $AgNO_3$ solution until light brick red precipitates appeared.

Calculations

Volume of N/40 $AgNO_3$ required for 5 ml of the sample = A ml

Volume of N/40 $AgNO_3$ required for blank = B ml

Amount of chlorides (me/L) =(A-B)x1x1000/5x40

2.4.6. Statistical analysis

To test the relative performance of genotypes and the significance of treatments the data were compared by the analysis of variance by using SAS software (9.4 version). Least significance difference (LSD) was calculated at 5% level of probability. Standard error (±) between the replications was also calculated.

3. Results

3.1. Osmoprotectants

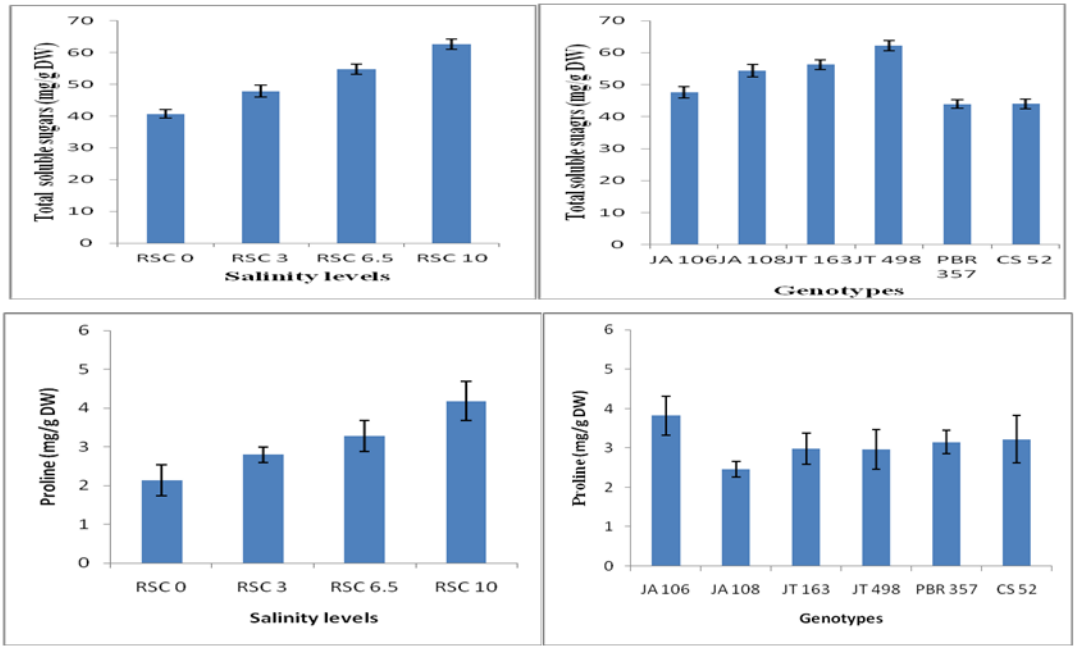
Plants counteract the stress-induced changes by regulating their defense systems like compatible solutes and antioxidants.

3.1.1. Total soluble sugars

Total soluble sugars improved under increased salinity by 14.9%, 25.6% and 34.9%with RSC 3, RSC 6.5 and RSC 10 respectively over non-saline condition. Accumulation of TSS was lowest in CS 52 as compared to other introgression lines under control and also with increased salinity. Sugar content was higher in JT 498 at RSC 6.5 and RSC 10 as compared to other ILs lines, released variety and even check. On an average JT498 assimilated higher total soluble sugars.

3.1.2. Proline

Mean proline content enhanced with the increase in salinity level to 23.6% with RSC 3, 34.7% with RSC 6.5 and 42.5% with RSC 10 over control (Table 1, Fig. 1).JA 106 accumulated more proline as compared to CS 52 at RSC 3 and RSC 6.5. However, proline content was higher in JT 163 and comparable in JA 106 and PBR 357 at salinity level (RSC 10). Genotypic average revealed minimum proline in JA 108 (2.46), comparable in JT 163 and JT 498 and higher in JA 106.



(A) (B)

Figure 1 Variation in total soluble sugars and proline with salinity levels (A) and genotypes (B)

3.2. Antioxidant molecules

Non-enzymatic antioxidants biomolecules protect from stress induced oxidative and ionic effects by minimizing the excess generation of ROS and hence protect cellular functioning.

3.2.1. Ascorbate

Under control conditions JT 163 possessed maximum vitamin C content and CS 52 1.67 (mg/g DW). Ascorbic acid increased in the introgression lines and the varieties with increased salt stress. Mean increase in ascorbic acid was 12.4% with RSC 3, 23.6% with RSC 6.5 and 28.9% with RSC 10 over control. Incremental increase in Vitamin C was maximum in JA 108 however; JT 498 had maximum content at RSC 10. On an average JA 108 had higher ascorbate content, comparable in JT 498 and PBR 357 and JT 163 and CS 52.

3.2.2. α-tocopherol

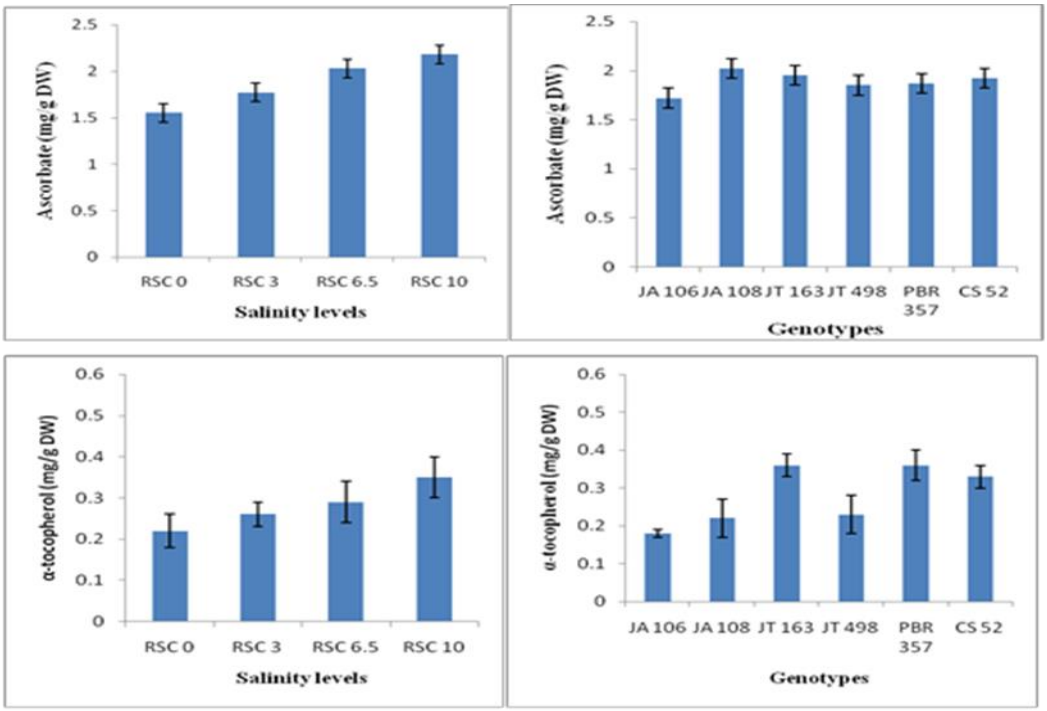
α-tocopherol, another antioxidant molecule increased with salinity to the tune of 15.4% with RSC 3, 24.1% with RSC 6.5 and 37.1% with RSC 10 over non saline treatment (Table 2, Fig. 2). Among the introgression lines JT 163had higher α-tocopherol (0.31 mg/g DW) under control. This line accumulated higher tocopherols (0.35 mg/g DW) while JA 106 and JA 108 lower content (0.19 mg/g DW) at salinity level RSC 3. α-tocopherol was relatively more in JT 163 (0.38 mg/g DW) and less in JA 106 (0.19 mg/g DW) with salinity treatment (RSC 6.5). Interestingly, JA 106 accumulated (0.19 mg/g DW) of tocopherols at RSC 3 and RSC 6.5. Higher salinity level of RSC 10, showed highest α-tocopherol accumulation in PBR 357 (0.54) as compared to check (0.37). On an average, tocopherol content was 0.18 in JA 106 to 0.36 mg/g DW in JT 163 and PBR 357.

3.3. Ionic estimation

Salinity had significant effect on ions and genotypes differed significantly for different ions studied. Interactions between salinity levels and genotypes (SxG) were also significant.

3.3.1. Calcium + Magnesium ions

Salinity significantly reduced [Ca^{2+}+ Mg^{2+}] in all introgression lines (ILs), PBR 357 (released variety) and salinity check (CS 52). Salinity levels reduced [Ca^{2+} + Mg^{2+}] ions by 14.5% with RSC 3, 28.3% with RSC 6.5 and 38.4% with RSC 10 over non saline treatment. [Ca^{2+} + Mg^{2+}] ions were maximum (2.11%) in JA 108 and minimum in (1.05%) JA 106 as compared to CS 52 under control. With RSC 3 salinity level, [Ca^{2+} + Mg^{2+}] concentration ranged from 0.91% (PBR 357 and JT 163) to 1.71% (JA 108) with respect to CS 52. JA 108 had 1.64% [Ca^{2+} + Mg^{2+}] as compared to CS 52 with salinity treatment of RSC 6.5. Lower content in JT 498 (0.65%) and maximum in JA 108 (1.51%) with higher salinity treatment of RSC 10. Average ionic content was 1.74% in JA 108 followed by JT 498 (1.1%) and 1.07% in CS 5.



(A) (B)

Figure 2 Variation in ascorbate and ά tocopherol with salinity levels (A) and genotypes (B)

3.3.2. Calcium ion

Uptake of Ca^{2+}introgressed lines along with the varieties in brassica decreased gradually with increased salinity levels. The Ca^{2+} ions were reduced with salt stress to 13.3% with RSC 3, 19.3% with RSC 6.5 and 34.9% with RSC 10 over control (Table 3). Introgression line JT 498 accumulated minimum and JA 108 maximum of Ca^{2+}ions at salinity level of RSC 3 and RSC 6.5 whereas higher residual sodium bicarbonate (RSC 10), accumulation of calcium ion again lesser in JT 498 (0.40%) and higher in CS 52 (0.71%). Genotypic average revealed lower Ca^{2+} ion in JT 498 and highest in CS 52.

3.3.3. Magnesium ion

Salinity stress significantly decreased Mg^{2+} ion with increased saline levels (Table 3). Mg^{2+}ions were reduced to 33.7% with RSC 3, 59.8% with RSC 6.5 and 81.5% with RSC 10 over non saline condition/ control. Mg^{2+} ions were maximum in JT 498 (1.54%) and minimum in JA 106 (0.31%). However, PBR 357 and CS 52 had similar Mg^{2+}content under control condition. Mg^{2+} was higher in JT 498 (1.01%) and lower in JA 106 (0.21%) as compared to check (CS 52) with RSC 3. Accumulation of Mg^{2+} content was lower in CS 52 (0.12%) which was almost similar to JT 163 (0.13%) and higher in JA 108 (0.71%) with salinity level of RSC 6.5. Similarly, with higher salinity level of RSC 10, Mg^{2+}ions were lower in JT 163 and CS 52 (0.11%) and higher in JT 498 (0.35%). Average of Mg^{2+}content in the introgression lines was 0.21% in JA 106 and 0.94% in JT 498.

3.3.4. Sodium ion

Presence of salt in the growing medium of the plant resulted in gradual increase of sodium ion uptake in plants (Table 4). The results were found to be highly significant for sodium ion uptake in all the ILs and varieties. With the increased

salinity Na^+ ions increased to 22.0% with RSC 3, 30.0% with RSC 6.5 and 39.3% with RSC 10 over non saline conditions. Minimum sodium accumulation was in JT 498 (0.58%), followed by JA 108 (0.71%) and maximum in JA 106 (0.95%) as compared to check (0.83%) with no added salt condition. Salinity level of RSC 3 lead to more Na^+ accumulation in JA 106 (1.05) and less in PBR 357 (0.76%). With increased salt stress (RSC 6.5) Na^+ ions were higher in PBR 357 (1.22%) followed by JA 106 (1.18%) and lower in JT 163 (1.00%) as compared to 1.13% in CS 52. Maximum accumulation of sodium ion was in JT 498 (1.39%) followed by PBR 357 (1.31%) and comparable in JA 106 and JT 163 (0.21%) as compared to check with higher salinity level of RSC 10. Three (JA 108, JT 163 and JT 498) ILs possessed 0.99% Na^+ ions whereas JA 106 had higher 1.09%

3.3.5. Potassium ion

Potassium uptake was necessary to tolerate saline environment. The potassium contents in the present investigations showed an overall decrease with increasing salinity level. K^+ ions uptake decreased by 7.6% with RSC 3, 14.4% with RSC 6.5 and 18.9% with RSC 10 over control or no salt added condition (Table 4). Minimum uptake of K^+ ion was 3.5% in JA 106 followed by 3.78% in PBR 357 whereas maximum uptake was 4.69% in JA 108 under non saline condition. K^+ ions were comparable in two varieties from 3.70% (JT 163) to 4.34% in JA 108 with salinity level of RSC 3. Similar trend was recorded in two varieties at higher salinity levels. Higher residual sodium bicarbonate treatment (RSC 10), registered maximum accumulation in JA 498 (3.53%) and minimum in JA 108 (3.02%). Genotypic average indicated lesser K^+ content in JA 106 as compared to check CS 52. Table 5 Effect of salinity on Na^+/ K^+ and K^+/Na^+ at flowering stage in Indian mustard genotypes.

3.3.6. Sodium /Potassium ratio

With increase in sodium ions accumulation and decrease in potassium ion contents resulted in increase of Na^+/ K^+ ratio in all ILs and varieties. The increase in salt stress mean was 10.3% with RSC 3, 21.2% with RSC 6.5 and 31.6% with RSC 10 over control (Table 5). Na^+/ K^+ ratio was highest (0.31) in JA 106 and lowest 0.22% in CS 52 in no added salt condition. With salinity level of RSC 3 minimum Na^+/ K^+ was in JA 108 (0.26), maximum in JA 106 (0.33) and comparable to check in JT 163 (0.28). Two ILs (JA 106 and JT 498) along with variety PBR 357 possessed similar ratio at RSC 6.5.At higher salt stress (RSC 10)Na^+/ K^+ was comparable in JT 498 and PBR 357 and comparable in ILs (JA 106 and JA 108). Average indicates comparable ratio in two ILs (JA 108 and JT 498) and CS 52 and JT 163**.**

3.3.7. Potassium / Sodium ratio

Introgression lines possessed higher K^+/Na^+ ratio as compared to varieties. Salt stress significantly decreased K^+/Na^+ ratio. The decline was 9.4% with RSC 3, 17.5% with RSC 6.5 and 20.7% with RSC 10 with non-saline treatment (Table 5). JT 498 had K^+/Na^+ ratio of 4.77 followed by 4.64 in JT 163. With salinity level of RSC 3, JA 106 had lowest K^+/Na^+(3.19) ratio while JT 498 had highest K^+/Na^+ of 4.04 as compared to check (3.71). Maximum K^+/Na^+ ratio was in JT 163 (3.57) and 3.22 in CS 52 with salinity treatment of RSC 6.5. Higher dose of salt stress revealed lowest (2.98) and highest (3.42) in ILs JA 106 and JA 108 respectively. On an average K^+/Na^+ was highest in JT 498 and least in JA 106 (Table 5).

3.3.8. Correlations

Total soluble sugar were highly negative correlated with seed yield at all the salinity levels whereas α-tocopherol has strong positive association with seed yield at RSC 10 and RSC 6.5 (r= 0.814*). Highly positive association existed between α-tocopherol at RSC 3 (r= 0.921**) and RSC 6.5 (r=0.898*) with ascorbate under control condition (Table 6). Seed yield was also positively correlated with proline at RSC 3 (r= 0.696) and RSC 6.5 (r= 0.714) with higher salinity treatment of RSC 10. Seed yield and Na^+/ K^+ were negatively correlated (Table 7) at RSC 6.5 (r= -0.934*). Na^+/K^+ ratio was positively correlated with K^+/Na^+ under control condition (r= 0.827*).

Table 1 Effect of salinity on total soluble sugars and proline in introgression lines and varieties of Indian mustard at flowering stage.

Total soluble sugars (mg/g DW)					**Average**	**Proline(mg/g DW)**				**Average**
Genotypes /treatment	RSC0	RSC3	RSC6.5	RSC10		RSC0	RSC3	RSC6.5	RSC10	
JA 106	31.55±0.5	45.75±3.6	53.32±1.2	60.13±1.9	47.68±1.8	2.07±0.3	4.11±1.6	4.46±2.6	4.65±2.2	3.82±0.5
JA 108	46.63±1.6	51.09±1.8	55.21±2.6	64.65±1.9	54.39±1.9	2.03±0.5	2.11±0.9	2.52±0.1	3.18±1.1	2.46±0.2
JT 163	51.41±1.2	53.24±1.5	55.75±1.5	64.72±1.7	56.28±1.5	1.75±0.1	2.24±0.5	3.10±1.3	4.84±0.5	2.98±0.4
JT 498	49.97±1.9	54.94±1.8	68.58±1.2	75.65±1.4	62.28±1.6	2.29±0.1	2.76±1.6	3.01±1.7	3.81±0.1	2.96±0.5
PBR 357	34.14±0.8	42.24±1.2	45.86±1.7	53.49±1.4	43.93±1.3	1.98±0.5	2.63±0.5	3.38±0.9	4.64±1.3	3.15±0.3
CS 52	30.36±1.8	39.54±1.3	49.53±1.5	56.70±1.5	44.03±1.5	2.72±0.6	2.95±0.3	3.22±0.8	3.99±1.4	3.22±0.6
Mean	40.67±1.3	47.80±1.9	54.70±1.6	62.55±1.6		2.14±0.4	2.80±0.2	3.28±0.4	4.18±0.5	
LSD (P=0.05)	S=17.14 G= 21.00 SxG=9.28					S= 1.36 G=1.66 SxG=2.61				

Table 2 Effect of salinity on ascorbate and α –tocopherol introgression lines and varieties at flowering stage.

Ascorbate (mg/g DW)					Average	α – tocopherol (mg/g DW)				Average
Genotypes/ Treatment	RSC0	RSC3	RSC6.5	RSC10		RSC0	RSC3	RSC6.5	RSC10	
JA 106	1.49±0.1	1.61±0.2	1.84±0.1	1.96±0.1	1.72±0.1	0.11±0.2	0.19±0.1	0.19±0.1	0.21±0.3	0.18±0.01
JA 108	1.34±0.2	2.13±0.2	2.26±0.5	2.34±0.4	2.02±0.1	0.18±0.1	0.19±0.2	0.23±0.2	0.29±0.1	0.22±0.05
JT 163	1.79±0.2	1.91±0.3	1.93±0.2	2.17±0.6	1.95±0.1	0.31±0.1	0.35±0.2	0.38±0.1	0.39±0.2	0.36±0.03
JT 498	1.33±0.2	1.56±0.2	2.06±0.4	2.45±0.6	1.85±0.1	0.17±0.2	0.21±0.3	0.22±0.1	0.33±0.1	0.23±0.05
PBR 357	1.71±0.2	1.77±0.1	1.96±0.6	2.04±0.1	1.87±0.1	0.24±0.1	0.29±0.1	0.37±0.1	0.54±0.5	0.36±0.04
CS 52	1.67±0.2	1.68±0.4	2.16±0.1	2.17±0.2	1.92±0.1	0.29±0.2	0.31±0.1	0.34±0.3	0.37±0.1	0.33±0.03
Mean	1.55±0.1	1.77±0.1	2.03±0.1	2.18±0.1		0.22±0.04	0.26±0.03	0.29±0.05	0.35±0.05	
LSD (P=0.05)	S= 0.36 G= 0.44 SxG=1.35					S= 0.17 G= 0.21 SxG=0.71				

Table 3 Effect of salinity on calcium and magnesium (Ca^{2+} + Mg^{2+}), calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions at flowering stage in Indian mustard.

Ca^{2+} + Mg^{2+}					**Average**	**Ca^{2+}**					**Mg^{2+}**				**Average**
Genotypes/ treatment	RSC0	RSC3	RSC6.5	RSC10		RSC0	RSC3	RSC6.5	RSC10	Average	RSC0	RSC3	RSC6.5	RSC10	
JA 106	1.05±0.2	1.00±0.1	0.97±0.1	0.70±0.1	0.93	0.78±0.1	0.61±0.1	0.58±0.3	0.48±0.2	0.61	0.31±1.4	0.21±0.1	0.19±0.3	0.12±0.1	0.21
JA 108	2.11±0.3	1.71±0.5	1.64±0.7	1.51±1.9	1.74	0.99±0.1	0.92±0.2	0.83±0.1	0.51±0.1	0.81	1.32±0.2	0.81±0.7	0.71±0.7	0.15±0.6	0.75
JT 163	1.10±0.5	0.92±0.7	0.87±0.6	0.77±0.1	0.92	0.82±0.2	0.80±0.2	0.76±0.1	0.68±0.2	0.77	1.02±0.9	0.59±0.5	0.13±0.4	0.11±0.5	0.46
JT 498	1.52±0.6	1.47±0.8	0.82±0.1	0.62±1.4	1.10	0.67±0.1	0.57±0.3	0.46±0.1	0.40±0.2	0.53	1.54±1.4	1.01±0.8	0.85±0.8	0.35±0.1	0.94
PBR 357	1.09±0.4	0.91±0.1	0.74±0.4	0.65±0.1	0.85	0.77±0.2	0.61±0.1	0.56±0.2	0.44±0.1	0.59	0.65±0.5	0.56±0.5	0.24±0.1	0.19±0.4	0.41
CS 52	1.46±0.6	1.09±0.1	0.91±0.1	0.82±1.4	1.07	0.97±0.1	0.81±0.1	0.79±0.3	0.71±0.1	0.82	0.65±0.5	0.50±0.2	0.12±0.3	0.11±0.4	0.35
Mean	1.38±0.4	1.18±0.4	0.99±0.3	0.85±0.8		0.83±0.1	0.72±0.2	0.67±0.2	0.54±0.2		0.92±0.8	0.61±0.5	0.37±0.4	0.17±0.4	
LSD (P=0.05)	S= 0.65 G= 0.80 SxG=1.81					S= 0.13 G=0.16 SxG=0.79					S= 0.69 G= 0.85 SxG=1.86				

Table 4 Effect of salinity on sodium (Na^{+}) and potassium (K^{+}) ions at flowering stage in Indian mustard

Na^{+} (%)					**Average**	**K^{+} (%)**				**Average**
Genotypes/ treatment	RSC0	RSC3	RSC6.5	RSC10		RSC0	RSC3	RSC6.5	RSC10	
JA 106	0.95±0.2	1.05±0.1	1.18±0.2	1.21±0.1	**1.09**	3.50±0.1	3.28±0.3	3.25±0.7	3.09±1.3	**3.28**
JA 108	0.71±0.2	0.95±0.3	1.04±0.3	1.25±0.2	**0.99**	4.69±0.5	4.34±0.4	3.53±0.6	3.02±1.1	**3.89**
JT 163	0.82±0.1	0.93±0.2	1.00±0.3	1.21±0.1	**0.99**	3.96±0.8	3.70±0.3	3.37±0.5	3.13±0.4	**3.54**
JT 498	0.58±0.1	0.99±0.3	1.03±0.1	1.39±0.1	**0.99**	4.07±0.1	3.86±0.9	3.60±1.2	3.53±0.5	**3.75**
PBR 357	0.72±0.1	0.76±0.2	1.22±0.1	1.31±0.2	**1.00**	3.78±0.3	3.43±0.7	3.30±0.9	3.25±0.6	**3.51**
CS 52	0.83±0.1	0.97±0.3	1.13±0.1	1.29±0.2	**1.05**	3.80±0.3	3.46±0.4	3.30±0.1	3.22±0.9	**3.44**
Mean	**0.77±0.1**	**0.94±0.2**	**1.10±0.2**	**1.27±0.2**		**3.96±0.4**	**3.66±0.5**	**3.39±0.6**	**3.21±0.8**	
LSD (P=0.05)	S= 0.19 G= 0.24 S x G=1.00					S=0.81 G=0.98 S x G=2.00				

Table 5 Effect of salinity on Na^+/ K^+ and K^+/Na^+ at flowering stage in Indian mustard

	Na^+/ K^+				Average	K^+/Na^+				Average
Genotypes/ treatment	RSC0	RSC3	RSC6.5	RSC10		RSC0	RSC3	RSC6.5	RSC10	
JA 106	0.31±0.2	0.33±0.1	0.34±0.1	0.45±0.2	0.36	3.27±0.2	3.19±0.9	3.00±0.1	2.95±1.5	3.10
JA 108	0.24±0.1	0.26±0.1	0.35±0.2	0.43±0.2	0.32	4.18±1.6	3.86±0.6	3.48±1.5	3.42±1.9	3.73
JT 163	0.26±0.2	0.28±0.1	0.29±0.1	0.33±0.1	0.29	4.64±2.2	3.88±1.4	3.57±0.3	3.09±0.6	3.79
JT 498	0.25±0.1	0.30±0.1	0.34±0.3	0.37±0.2	0.32	4.77±3.1	4.04±0.4	3.54±0.9	3.27±0.3	3.90
PBR 357	0.29±0.1	0.32±0.2	0.34±0.1	0.36±0.2	0.33	3.66±0.9	3.39±0.2	3.20±1.3	3.11±0.2	3.34
CS 52	0.22±0.1	0.28±0.1	0.32±0.1	0.34±0.1	0.29	3.83±0.7	3.71±0.8	3.22±0.3	3.12±0.6	3.47
Mean	0.26±0.1	0.29±0.1	0.33±0.2	0.38±0.2		4.05±1.5	3.67±0.7	3.34±0.7	3.21±0.8	
LSD(P=0.05)	S=0.13 G=0.16 SxG=0.79					S=1.64 G=2.02 SxG=2.87				

Table 7 Correlation coefficient between Na^+/K^+ and K^+/Na^+ with seed yield.

Parameters / salinity levels		**Na^+/K^+**				**K^+/Na^+**				**Seed yield**			
		RSC0	RSC 3	RSC 6.5	RSC 10	RSC0	RSC 3	RSC 6.5	RSC 10	RSC0	RSC 3	RSC 6.5	RSC 10
Na^+/K^+	RSC0	1											
	RSC 3	0.850*	1										
	RSC 6.5	0.193	0.240	1									
	RSC 10	0.444	0.230	0.708	1								
K^+/Na^+	RSC0	-0.265	0.010	0.827*	0.313	1							
	RSC 3	-0.773	-0.748	-0.250	-0.431	0.164	1						
	RSC 6.5	-0.558	-0.687	-0.319	-0.405	-0.050	0.933**	1					
	RSC 10	-0.619	-0.714	0.386	0.083	0.544	0.713	0.673	1				
Seed yield	RSC0	0.523	0.636	0.236	0.185	-0.031	-0.919	-0.934**	-0.634	1			
	RSC 3	0.404	0.331	-0.427	-0.545	-0.505	-0.075	0.182	-0.304	0.066	1		
	RSC 6.5	0.430	0.325	-0.380	-0.477	-0.553	-0.307	-0.057	-0.387	0.349	0.928**	1	
	RSC 10	0.718	0.578	0.547	0.367	0.132	-0.596	-0.388	-0.091	0.524	0.432	0.532	1

*significant at 5% level **significant at 1% level

Table 6 Correlation coefficient between total soluble sugar, proline, ascorbate, tocopherols with seed yield at different salinity levels.

Parameters/ salinity levels		Total soluble sugar				Proline				Ascorbate				α- tocopherols				Seed yield			
		RSC0	RSC 3	RSC 6.5	RSC 10	RSC0	RSC 3	RSC 6.5	RSC 10	RSC0	RSC 3	RSC 6.5	RSC 10	RSC0	RSC 3	RSC 6.5	RSC 10	RSC 0	RSC 3	RSC 6.5	RSC 10
TSS	RSC0	1																			
	RSC 3	0.941**	1																		
	RSC 6.5	0.711	0.831*	1																	
	RSC 10	0.793	0.889*	0.990**	1																
Proline	RSC0	-0.480	-0.498	0.010	-0.068	1															
	RSC 3	-0.660	-0.384	-0.093	-0.205	0.261	1														
	RSC 6.5	-0.631	-0.391	-0.260	-0.355	-0.019	0.932**	1													
	RSC 10	-0.247	-0.215	-0.351	-0.390	-0.395	0.369	0.655	1												
Ascorbate	RSC0	-0.258	-0.442	-0.637	-0.628	-0.169	-0.097	0.179	0.751	1											
	RSC 3	0.396	0.228	-0.230	-0.094	-0.471	-0.711	-0.618	-0.365	0.013	1										
	RSC 6.5	0.189	0.027	0.105	0.164	0.455	-0.576	-0.802	-0.910*	-0.412	0.488	1									
	RSC 10	0.722	0.652	0.765	0.805	0.194	-0.585	-0.790	-0.743	-0.591	0.182	0.675	1								
α-tocopherols	RSC0	0.111	-0.193	-0.338	-0.290	0.054	-0.574	-0.420	0.253	0.784	0.246	0.150	0.001	1							
	RSC 3	0.003	-0.248	-0.398	-0.374	-0.051	-0.363	-0.144	0.543	0.921**	0.065	-0.164	-0.235	0.949**	1						
	RSC 6.5	-0.041	-0.327	-0.531	-0.495	-0.113	-0.476	-0.238	0.467	0.898*	0.193	-0.075	-0.248	0.929**	0.953**	1					
	RSC 10	-0.053	-0.305	-0.431	-0.417	-0.114	-0.486	-0.289	0.314	0.620	0.073	-0.031	-0.115	0.650	0.665	0.831*	1				
Seed yield	RSC0	-0.938**	-0.887*	-0.807	-0.874*	0.171	0.594	0.675	0.437	0.378	-0.297	-0.378	-0.859*	-0.106	0.054	0.151	0.220	1			
	RSC 3	0.219	0.099	-0.179	-0.162	-0.606	-0.291	0.023	0.696	0.633	-0.011	-0.554	-0.262	0.436	0.594	0.667	0.758	0.066	1		
	RSC 6.5	-0.061	-0.211	-0.530	-0.507	-0.548	-0.237	0.098	0.714	0.771	0.110	-0.502	-0.503	0.495	0.648	0.768	0.814*	0.349	0.928**	1	
	RSC 10	-0.256	-0.199	-0.386	-0.391	-0.473	0.106	0.258	0.222	-0.020	0.074	-0.327	-0.418	-0.380	-0.272	-0.033	0.368	0.524	0.432	0.532	1

*significant at 5% level ** significant at 1% level

4. Discussion

Osmoprotectants, antioxidant molecules and role of ions are the backbone of any plant, so if the changes in these attributes occur on being exposed to any type of stress, it can be used as a good stress marker. In the present study we found the variability among the introgression lines and varieties of mustard in response to different saline levels. Compatible solutes included amino acids, glycerol, sugars and other low molecular weight metabolites, served to re-establish osmotic homeostasis by increasing water potential in response to osmotic stresses. In our study, osmoprotectants (total soluble sugars and proline) and antioxidant molecules (ascorbate and α-tocopherol) were enhanced with increased salinity levels. Increase in concentration of total soluble sugars in safflower genotypes were reported [18]. Soluble sugars may act as osmoprotectants for protein under stressed condition [19]. Similar results were reported [20] in *Nitrariatun gutorum*. Accumulation of soluble sugars as a consequence of salt stress may partially explain the mildly tolerant behavior of *Brassica* towards salt stress. Proline and glycine betaine were also reported to accumulate under salt stress in *B. juncea* [21], Linseed [22] and mulberry [23]. A higher content of proline in response to increased salinity has been observed in salt tolerant cultivars of sunflower, finger millet and rice [24, 25].

It has been proposed that plants can activate ascorbic acid for protection in the initial periods of stress before the expression of antioxidant enzymes to clear some of the active oxygen and reduce damage, which also lower the contents of MDA [26]. According to [27] highest ascorbic acid content was observed in bay leaf and lowest in poppy seeds. Further, in tomato increased ascorbate content with increasing salinity in the growth medium was reported [28]. However, contradictory results were observed in mungbean [29] and in wheat [30] where reverse trend was reported with increased salinity. Ascorbic acid content was reduced as observed in *Linum usitataissimum* plants [31] and in Amaranthus polygamous [32]. Salinity stress also increased α-tocopherol in safflower [33] and in sunflower [34]. Ascorbate and tocopherol content increased in leaves of tomato to protect them against oxidative stress under triazole treatment as well as in wheat leaves under higher salinity as reported [35].

Introgression lines along with the variety and CS 52, salinity check exhibited significant differences in the ion accumulation in leaves under different salinity treatments. Increasing level of salinity lead to elevated concentration of Na+ in leaves as compared to control. The higher Na^+ accumulation can be attributed to the differential cellular entry of ions under high salinity, as the similarity in the hydrated ionic radii between Na^+ and K^+ makes it difficult for the transporter to discriminate between two ions. Increased levels of salinity lead to reduction in K^+ content in leaves. The enhanced content K^+ might have contributed to the cellular level salt tolerance .In the vacuoles the harmful ions are compartmentalized for intracellular ion homeostasis which is necessary for cytoplasmic metabolic activity and there is an increase in cellular osmolarity to counter osmotic stress. Salt stress imposes an ionic imbalance by elevating Na^+ at the expense of K^+ and Ca^{2+} depletion [36]. Na^+ and K^+ can be used as a screening tool under saline regimes [37, 38]. According to our research, availability of all the ions reduced with increased with increased salinity levels, except Na^+. With increase Na^+ ion, Na^+/K^+ ratio increased while K^+ and K^+/Na^+ decreased. Ionic content, among the introgression lines and varieties revealed low Na^+ and high K^+ correspondingly Na^+/K^+ low and K^+/Na^+ ratio higher. It has been reported by some researchers that salinity cause significant reduction in Ca2+ ion accumulation in plants [39]. Decrease in $Ca^{2+}$and Mg^{2+} contents in the leaves upon salinity stress suggested increased membrane stability and decreased chlorophyll content [40]. Salinity stress increased Na^+ content as reported in Brassica cultivars [41]. High Na^+ content generally disrupted the nutrient balance causing specific ion toxicity despite disturbing osmotic regulation. Potassium content decreased with increase in salt stress. These findings were reported in soybean [42], green bean [43] and canola cultivars [7]. Increased sodium ion and decreased potassium leads to reduction in K^+/Na^+. Salinity tolerance is related to the ability of plant to maintain lower Na^+/K^+ ratio [44] rather than simply maintaining lower Na^+ concentration. Higher salinity stress caused high sodium uptake and increased Na^+/K^+ ratio values cause sodicity, which increase soil resistance, reduces root growth and reduces water movement through the root with a decrease in hydraulic conductivity [45] and its reduction depends upon the salt content of the irrigation water used thus affecting the level of water absorption 46] maintaining ion homeostasis by ion exclusion and compartmentalization is an essential process for growth during salt stress. Controlled Na^+ uptake and lower Na^+/K^+ reduced the toxic effect of Na^+ in the cytosol and increasing cell water uptake [47]

Tomato plants under salt stress exhibited a decrease of K^+/ Na^+ ratio in both leaves and mature fruits. Accumulation of Na ions increased with rise in NaCl concentration. [48] reported increased accumulation of Na^+ in soybean under salt stress. Agglomeration of Na^+ in salt tolerant and salt susceptible varieties of azolla is reported [49]. Building up of Na ions with increased salt stress was reported [50] in tomato. Na^+ competes with K^+ ions uptake because both elements have similar physio-chemical structure. Increased salt content leads to sodium accumulation and lowers potassium leading to disrupted K^+/ Na^+ ratio as reported by [51]. K^+ has an important role in osmotic adjustment in the guard cell controlling the stomata movement and thus CO_2 assimilation in photosynthesis [52]. Moreover, K^+ is considered to be an effective agent in plant salt tolerance mechanisms through maintenance of Na^+/K^+ homeostasis [53] and

osmoregulation [54]. Accumulation of inorganic ions for osmotic adjustment is an energy effective way for higher plants to combine productivity with salt tolerance [55]. The growth could be inhibited by reduction in K^+ concentration which reducing the capacity for osmotic adjustment and turgor maintenance or adversely affecting metabolic functions [56]. Turgor may be maintained by the help of Na^+. Na^+, however is unable to substitute for specific functions of Ca^{2+} and K^+ particularly enzyme activation and protein synthesis to produce adequate growth. Increasing Na^+ contents and decreasing K^+ contents and K^+/Na^+ ratios in plant leaves can be attributed to the effect of competition between Na^+ and K^+ ions on the absorptive sites of the plant roots [57].A non-significant positive correlation was exhibited between seed yield and K whereas significant negative correlation between K^+ and Na^+ in rapeseed.

5. Conclusion

Average yields were relatively higher in the introgression lines JT 163 (6.4%) and PBR 357 (16.5%) over national check CS52 rating them tolerant to salt stress. The better performance was attributed to higher osmoprotectants, ant oxidative molecules and higher concentration of Na^+/K^+ ion with lesser damage to membrane stability added with higher tolerance index (0.82) and lower susceptibility index (0.60).

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Author Contributions

PS conceived, designed the experiments, KP performed the biochemical analysis, OPC provided the fields for the experiments and resources, PS and VS supervised the work, PS reviewed and edited the manuscript.

References

[1] Zaman MM, Mannan MA, Samanta SC. Salt induced changes in dry matter accumulation and yield of mustard. Bangladesh Argon J. 2015; 18: 65-75.

[2] Rani B, Kumar R, Gupta PC. Increasing oilseed production through cluster demonstration in Patna district. A case study. J Oilseed Brassica. 2018; 9: 65-87.

[3] Patel R, Yadav RC, Avtar R, Jangra S, Boken GD, Singh B, Yadav NR. Genetic diversity analysis for salinity tolerance in Indian Mustard [*Brassica juncea*(L)] using SSR markers. Int. J Curr. Microbial. App Sci. 2018; 7: 1776-1785.

[4] Ahanger MA, Agrawal RM. Salinity stress induced alternations in antioxidant metabolism and nitrogen assimilation in wheat (*Triticum aestivum* L.) as influenced by potassium supplementation. Plant Physiol. Biochem. 2017; 115: 449-460.

[5] Jat AL, Prajapati KP, Patel BK, Patel PJ, Patel JR, Shah SK, Desai AG. Influence of NPK levels on performance of Indian Mustard [*Brassica juncea*(L)]. Int J Curr Microbiol App Sci. 2017; 6: 1986-1990.

[6] Lang F. Mechanisms and significance of all volume regulations. J Am Coll. Nutr. 2007; 26: 613-623.

[7] Bjelakovic G, Nikolova D, Gluud C. Meta–regression analyses, meta- analyses, and trail sequential analyses of the effects of supplementation with beta- carotene, Vitamin A and Vitamin E singly or in different combinations on all-cause mortality. PLoS one. 2013; 8: e74558.

[8] Levin M. New concepts in the biology and biochemistry of ascorbic acid. New Eng J Med. 1986; 31: 892-902.

[9] Liebler DC. The role of metabolism in the antioxidant function of Vitamin E. Critical Rev Toxicol. 1993; 23: 147-169.

[10] Manzer HS, Firoz M, Khan MN. Morphological and physio-biochemical characterization of *Brassica juncea*L. Czern. & Coss genotypes under salt stress. J Plant Inter. 2009; 4: 67-80.

[11] Khan MN, Siddiqui MH, Mohammad F, Khan MMA, Naeem M. Salinity induced changes in growth, enzyme activities, photosynthesis, proline accumulation and yield in linseed genotypes. World J Agri. Sci. 2007; 3: 685-695.

[12] Parveen S, Shahbaz M, Ashraf M. Changes in mineral composition, uptake and use efficiency of salt stressed wheat (*Triticum aestivum*L.) plants raised from seed treated with triacontanol. Pak J Bot.2012; 44: 27-35.

[13] Pandey M, Penna S. Time course of physiological, biochemical and gene expression changes under short-term salt stress in *Brassica juncea* L. The Crop J. 2017; 219-230.

[14] Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem. 1956; 28: 350-356.

[15] Bates LS. Rapid determination of free proline content for water stress studies. Plant Soil. 1973; 39: 205-257.

[16] Roe JH,Kuether CA. The determination of ascorbic acid in whole blood and urine through the 2, 4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J Biol. Chem. 1943; 147: 399-307.

[17] Asthir B, Kaur S, Mann SK. Effect of salicylic and abscisic acid administered through detached tillers on antioxidant system in developing wheat grains under heat stress. Acta Physiol. Plant. 2009; 31: 1091-1096.

[18] Javed Q, Wu Y, Xing D, Azeem A, Ullah I, Zaman M. Re-watering: An effective measure to recover growth and photosynthetic characterstics on salt- stressed B. napus L. Chilean J Agri. Res. 2017; 77: 55-76.

[19] Ashraf MY, Ashraf M, Sarwar G. Response of Okra (*Hibiscus esculentus*) to drought and salinity stress. In vegetables: growing environment and mineral nutrition (Ed): R. Dres. 2005; 166-77.

[20] Liu W, Zhang Y, Yaun X, Xuam X, Gao Y, Yan Y. Exogenous salicylic acid improves salinity tolerance of *Nitrariatun gutorum*. Russ J Plant Physiol. 2016; 63: 132-142.

[21] Khan MN, Siddiqui MH, Mohammad F, Naeem M. Interactive role of nitric oxide and calcium chloride in enhancing tolerance to salt stress. Nitric Oxide. 2012; 27: 210-218.

[22] Khan MN, Siddiqui MH, Mohammad F, Naeem M, Khan MMA. Calcium chloride and gibberellic acid protect linseed (*Linum usitatissiumL.*) from NaCl stress by inducing antioxidant defence system and osmoprotectant accumulation. Acta Physiol. Plant. 2010; 32: 121-132.

[23] Ahmad P, Ozturk M, Sharma S, Guce S. Effect of sodium carbonate-induced salinity- alkalinity on some key osmoprotectants, protein profile, antioxidant enzymes and lipid per oxidation in two Mulberry (*Morus alba* L.) cultivars. J Plant Interact. 2014; 9: 460-467.

[24] Mittal S, Kumari N, Sharma V. Differential response of salt stress on *Brassica juncea*photosynthetic performance, pigment proline, D1 and antioxidant enzymes. Plant Physiol. Biochem. 2012; 54: 17-26.

[25] Kibria MG, Hossain M, Murata Y, Hoque MA. Antioxidant defense mechanisms of salinity tolerance in rice genotypes. Rice Sci. 2017; 24: 155-162.

[26] Wang HM, Xiao XR, Yang MY , GaoZL, ZangJ, Fu XM, Chen YH. Effects of salt stress on antioxidant defense system in the root of *Kandelia Candel*, Bot. Stud. 2014; 55: 57.

[27] Dinesh B, Yadav B , Reddy D ,Padma AS, Sukumaran MK. Determination of ascorbic acid content in some Indian mustard species. Int. J Curr. Microbiol. App Sci. 2015; 4: 864-868.

[28] Gautier H, Lopez-Lauri F, Massot C, Murshed R , Marty I , Graselly D , Keller C , SallanonH, Genard M . Impact of ripening and salinity on tomato fruit ascorbate content and enzymatic activities related to ascorbate recycling. Functional Plant Sci. Biotech. 2010; 4: 66-75.

[29] Asharf MA, Ashar fM,Shahbaz M. Growth stages based modulation in antioxidant defense system and proline accumulation in two hexaploid wheat (*Triticum aestivum*L.) cultivars differing in salinity tolerance. Flora. 2012; 207: 388-397.

[30] Kanwal S , Ashraf M, Shahbaz M, Iqbal MY. Influence of saline stress on growth, gas exchange, mineral nutrients and non-enzymatic antioxidants in mungbean [*Vigna radiata*(L.) Wilczek] Pak .J. Bot. 2013; 45: 763-771.

[31] Emam MM, Helal NM. Vitamins minimize the salt-induced oxidative stress hazards. Aust. J Basic Appl. Sci. 2008; 2: 1100-1119.

[32] Ratnakar A, Rai A. Effect of NaCl salinity on β-carotene, thiamin, riboflavin and ascorbic acid contents in the leaves *Amaranthus polygamous*L. var. Pusa Kirti. Oct J Env. Res. 2013; 1: 211-216.

[33] Siddiqi EH, Ashraf M, Al-Qurainy F, Akram NA. Salt-induced modulation in inorganic nutrients, antioxidant enzymes, proline content and seed oil composition in safflower (*Carthamus tinctorius*L.). J Sci. Food Agric. 2011; 91: 2785-2793.

[34] Noreen S, Ashraf M. Modulation in salt (NaCl) induced effects on oil-composition and fatty-acid profile of sunflower (*Helianthus annuus*L.) by exogenous application of salicylic acid. J Sci Food Agric. 2010; 90: 2608-2616.

[35] Tuna AL. Influence of foliar applied different triazole compounds on growth, nutrition and antioxidant enzyme activities in tomato (*Solanum lycopersicum*L.) under salt stress. Aus. J Crop Sci. 2014; 8: 71-79.

[36] Morgan SH, Maity PJ, Geilfus CM, Lindberg S, Muhling KH. Leaf ion homeostasis and plasma membrane H+-ATPase activity in *Vicia faba*change after extra calcium and potassium supply under salinity. Plant Physiol. Biochem. 2014; 82: 244-253.

[37] Chen ZH, Zhou MX, Newman IA, Mendham NJ, Zhang GP, Shabala SN. Potassium and sodium relations in salinised barley tissues as a basis of differential salt tolerance. Functional .Plant. Biol. 2007; 34: 150-162.

[38] Cuin TA, Tian Y, Betts SA, Chalmandrier R, Shabala S. Ionic relations and osmotic adjustment in durum and bread wheat under saline conditions. Functional Plant. Biol. 2009; 36: 110-119.

[39] Haleem A, Mohammed MA. Physiological aspects of mungbean plant (*Vigna radiata*L. Wilczek) in response to salt stress and gibberellic acid treatment. Res J AgricBiolSci. 2007; 3: 200-213.

[40] Parida AK, Das AB. Salt tolerance and salinity effects on plants: a review. Ecotoxicol Environ Saf. 2005; 60: 324–342.

[41] Rameeh S, Rezai A, Saeidi G. Study of salinity tolerance in rapeseed. Commun Soil Sci. Plant Anal. 2004; 35: 2849-2866.

[42] Li X, An P, Inanaga S, Eneji AE, Tanabe K. Salinity and defoliation effects on soybean growth. J Plant Nutr.2006; 29: 1499-1508.

[43] Yasar F , Uzal O , Tufenkci S, Yildiz K. Ion accumulation in different organs of green bean genotypes grown under salt stress. Plant Soil Environ. 2006; 52: 476-480.

[44] Almeida DM, Oliveira MM, Saibo NJM. Regulation of Na^+ and K^+homeostasis in plants: towards improved salt stress tolerance in crop plants. Genet. Mol. Biol. 2017; 40(1): 326-345.

[45] Singh J, Singh V, Vineeth TV, Kumar N, Kumar P, Sharma PC. Differential response of Indian mustard (*Brassica juncea*L. Czern and Coss) under salinity: Photosynthetic traits and gene expression. Physiol. Mol. Biol. Plants.2018; 25(1):71-83.

[46] Zobel RW, Kinraide TB, Baliger VC. Fine root diameter can change in response to changes in nutrient concentrations. Plant Soil. 2007; 297: 243-253.

[47] Singh J, Singh V, Sharma PC. Elucidating the role of osmotic, ionic and major salt responsive transcript components towards salinity tolerance in contrasting chickpea (*Cicer arietinum*L.) genotypes. Physiol.Mol..Biol. Plants. 2018; 24(3): 441-453.

[48] Kaolo Y , Tsai TT, Tsai HC, Shih CN. Response of three glycine species to salt stress. Environ Exp Bot. 2006; 56: 120-125.

[49] Masood A, Shah NA, Zeeshan M, Abraham G. Differential response of antioxidant enzymes to salinity stress in two varieties of Azolla (*Azollapinnata* and *Azollafiliculoids*).Environ Exp Bot. 2006; 58: 216-222.

[50] Maggio A , Raimondi G, Martino A, De Pascale S. Salt stress response in tomato beyond the salinity tolerance threshold. Environ Exp Bot. 2008; 59: 276-282.

[51] Zhang JL, Flowers TJ, Wang SH. Mechanism of sodium uptake by roots of higher plants. Plant Soil. 2010; 326: 45-60.

[52] Mass EV. Crop salt tolerance, in Agricultural Salinity Assessment and Management , ed.by Tanji K K.ASCE, Reston. 1990; 262-305.

[53] Aleman F, Nieves- cordones M, Martinez V, Rubio F. Potassium/sodium steady-state homeostasis in *Thellungiella halophila* and *Arabidopsis thaliana*under long-term salinity conditions. Plant Sci. 2009; 179: 768-774.

[54] Orsini F, Accorsi M, Gianquinto G, Dinelli G, Antognoni F, Carrasco KBR. Beyond the ionic and osmotic response to salinity in *Chenopodium quinoa*: Functional elements of successful halophytism. Functional Plant Biol. 2011; 38: 818-831.

[55] Yeo AR, Flowers TJ. Accumulation and localization of sodium ions within the shoots of rice (*Oryza sativa*) varieties differing in salinity resistance. Physiol. Plant arum. 1982; 56: 343-348.

[56] Greenway H, Munns R. Mechanisms of salt tolerance in non halophytes. Ann Rev Plant Physiol. 1980; 31: 149-190.

[57] Bhivare VN, Nimbalkar JD. Salt stress effects on growth and mineral nutrition of French beans. Plant Soil. 1984; 80: 91-98.